REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-5, 8, 9 and 11-21 are in this case. Claims 11-19 were withdrawn under a restriction requirement as drawn to a non-elected invention. Claims 1-5, 8, 9, 20 and 21 have been rejected. Claim 9 has now been cancelled. New claim 22 has now been added. Claims 1-5, 8 and 20-21 have now been amended.

35 U.S.C. § 112, First Paragraph, Rejections

The Examiner has rejected claims 1-5, 8, 9 and 20-21 under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. The Examiners rejections are respectfully traversed. Claim 9 has now been cancelled. New claim 22 has now been added. Claims 1-5, 8 and 20-21 have now been amended.

The Examiner states that claims 1, 5, 8 and 21 recite limitations which are not supported by the specification.

The Examiner states that claims 1, 5 and 8 relate to a "chelating marker" which is not literally or contextually supported by the specification. Claims 1, 5 and 8 have now been amended and the phrase "chelating marker" has now been replaced by the term --complex-- which is described and exemplified on the last paragraph of page 6 of the published PCT application (WO 00/36422).

The Examiner also states that the phrase "wherein said non-bound metal ion and said additional ion are different" of claim 21 is not literally or contextually supported by the specification.

Claims 1, 2, 20 and 21 have been amended to replace the phrase "non-bound metal ion" with "non-bound iron". This limitation is clearly supported in the specification of the instant application (see e.g., Page 7 4th paragraph of the published PCT application (WO 00/36422).

Claim 21 has now been amended and the above recited phrase replaced with "wherein said non-bound iron in the sample of biological fluid and said metal ion of said complex are of different type".

This limitation is clearly supported by Example 4 which describes determination of Aluminum sample concentrations using DFO and the calcein marker (which includes iron, a metal ion of "different type").

In view of the above arguments and claim amendments, Applicant believes to have overcome the 35 U.S.C. §112, first paragraph, rejections.

35 U.S.C. §112, Second Paragraph, Rejections

The Examiner has rejected claims 1-5, 8, 9 and 20-21 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. The Examiners rejections are respectfully traversed. Claim 9 has now been cancelled. Claims 1-5, 8 and 20-21 have now been amended. New claim 22 has now been added.

With respect to claim 1, the Examiner points out that the recitation of "an additional metal ion" is indefinite because it is not clear whether this metal ion is derived from the sample.

Claim 1 has now been amended to recite a "complex comprising a marker bound to a metal ion" clearly identifying this metal ion as a part of the complex and distinguishing it from the non-bound iron of the sample.

The Examiner further states that Step (b) of claim 1 appears to contradict Step (c) and step (d), in that step (b) recites a "chelating marker chelating an additional metal ion", whereas steps (c) and (d) each recite a "chelating marker not chelating said additional metal ion.

As is clearly stated in the specification, the present method enables quantification of non-bound iron ions of a sample by exposing such iron ions to a surface-conjugated chelator (of known binding capacity) and subsequently quantifying the unbound sites of this surface-conjugated chelator. The latter quantification is effected by using a complex which includes a marker bound to a metal ion (of a type identical to, or different from the non-bound metal ion of the sample). By exposing the surface-conjugated chelator to this complex, any unbound surface-conjugated chelator sites will chelate the metal ion of the complex releasing

non-metal ion bound marker molecules (i.e., dissociated marker molecules) which can be quantified. The quantity of such "free" marker molecules directly correlates with the number of unbound surface-conjugated chelator sites and thus provides a measure of the number of bound surface-conjugated chelator sites and of the quantity and concentration of non-bound iron in the sample (see attached scheme).

Thus, the present method utilizes a single marker complex which can exist in a first state wherein it is chelating said additional metal ion and a second state wherein it is not chelating said additional metal ion.

In the interest of clarity, Applicant has elected to amend claim 1 to replace "chelating marker chelating an additional metal ion" with --a complex comprising a marker bound to a metal ion-- and "chelating marker not chelating said additional metal ion" with --said marker dissociated from said metal ion--.

With respect to claim 3, the Examiner states that it is not clear whether desferrioxamine (DFO) is a polymer-conjugated chelator or whether a separate polymer is conjugated to DFO.

Claim 3 has now been amended to recite "wherein said polymer-conjugated metal chelator is desferrioxamine (DFO)" clearly stating that DFO is a polymer-conjugated chelator.

35 U.S.C. §103(a) Rejections

The Examiner has rejected claims 1-2, 5, 8-9 and 20 under 35 U.S.C. §103(a) as being unpatentable over Skold et al. in view of Breur et al. The Examiner's rejections are respectfully traversed. Claim 9 has now been cancelled. Claims 1-2, 5, 8 and 20 have now been amended. New claim 22 has now been added.

As is detailed above and in the specification, the present invention relates to a method of indirectly quantifying non-bound iron present in a biological sample. The attached Figure 1a shows the main features of the present invention in a stepwise manner. Step a: A biological fluid is contacted with a surface coated with a polymer-conjugated iron chelator under conditions suitable for chelation of the non-bound iron present in the sample;

Step b: The surface resulting from step a, now having iron ions chelated thereon, is contacted with a complex which includes a marker bound to a metal ion, under conditions which allow an exchange of the metal ion from the marker to surface-bound iron-free-chelator (unoccupied chelator); Step c: Determining the amount of dissociated marker molecules (free/unbound), which is indicative of the concentration of the non-bound iron in the sample (inversely correlated). Preferably, in its unbound state, the marker fluoresces.

The Examiner states that Skold et al essentially teaches the method of the present invention but fails to teach a chelating marker chelating an additional metal ion. Applicant fails to see the relevance of this reference since it is this (chelating) marker chelating the metal ion which is central to the present detection method. As is mentioned hereinabove, the dissociation of this complex enables indirect detection of non-bound chelator sites via quantification of metal ion-free marker molecules. Even if the Examiners assertion that Skold et al. indeed measure non-bound sites (see attached Figure 1b), such measurements are effected directly and not through an exchange of a metal ion from a marker complex to a non-bound site of a surface-conjugated chelator. In fact, the present invention quantifies molecular events, while Skold et al. teaches surface adsorption. It should be noted in this respect, that measuring non-bound iron using indirect means, as taught and claimed by the instant application, is highly advantageous over the teachings of Skold et al., as it significantly reduces background signal and as such contributes to the accuracy of the assay. Specifically, Skold et al. teach direct binding of the marker (analyte analog) to the surface and as such measure also non-specific binding of the marker to the surface. To overcome this limitation, the present invention teaches determining the amount of dissociated marker (free/unbound in solution) and as such increases accuracy of testing immensely.

Thus, Skold et al. and the present inventors take a completely different approach to measuring non-bound metal ions.

Although Breur et al. utilize calcein to measure metal ion concentrations in cells, such measurements are direct in that calcein is utilized to capture non-bound metal ions and not to exchange a metal ion bound thereto with a surface-conjugated

chelator. The purpose of the study conducted by Breur et al. was to determine metal ion transport using calcein as a direct measurement probe. Breur et al. do not mention or propose use of this probe in a multi-component measurement system designed for quantifying non-bound metal ions present in a biological sample. In fact, the Breur et al. study merely utilizes calcein as a tool for deciphering the transport of iron and other transition metals in cells and thus no mention of the suitability or applicability of this probe for diagnostic purposes is made.

Thus, it is Applicant's strong opinion that the combined teachings of Skold et al. and Breur et al. do not render obvious the present invention and cannot be used as a basis for obviousness type rejections when used in combination with Yegorov et al. or Guire and Chudzik.

In view of the above amendments and remarks it is respectfully submitted that claims 1-5, 8 and 20-22 are now in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,

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Encl.

1. Two-Month Extension Fee;
2. Figures 1a-b